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Aug 30, 1968

POSSIBLE CIRCADIAN RHYTHM IN *Endothia parasitica*

A THESIS

Presented to

The Faculty of the Graduate Division

by

Daniel Morgan Raiford

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in The School of Applied Biology

Georgia Institute of Technology

August, 1968

POSSIBLE CIRCADIAN RHYTHM IN *Endothia parasitica*

Approved:

Chairman

Date approved by Chairman: 8/21/68

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to Professor T. W. Kethley for his guidance not only in the preparation of this thesis but also during my entire graduate program. My appreciation is extended to Dr. John J. Heise and Dr. Edward K. Yeargers for their assistance. I am grateful to Mrs. Joyce Williams for her secretarial assistance. This thesis is dedicated to Wilma Louise Hodnett whose assistance made it possible.

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SUMMARY

The objective of this research was to determine the effect of light at different wavelengths, on a possible circadian rhythm in the colonial growth of the causative agent of the chestnut tree blight, Endothia parasitica. Previous evidence suggested that light influences circadian rhythmic growth (or zonation) and that a particular wavelength region was the responsible stimulus.

Zone formation was studied under three main conditions; total darkness, intermittent light, or continuous light. Four light sources were employed, ranging from sunlight to a narrow beam band of ultraviolet light in the 365 nm range. Cultural growth experiments were conducted for eight days under the above conditions.

It was concluded that zonation in E. parasitica does not appear to be related to any endogenous circadian rhythm, but does appear to be affected by light. A lamp whose output wavelength peaks at 375 nm has a particular stimulating effect on matrix growth but acts as an inhibitor for above surface growth, such as aerial hyphae. It is not possible, however, to be certain that the 375 nm region is the one responsible because of the uncertainty in absolute spectral intensities of the lamp at various wavelengths.

CHAPTER I

INTRODUCTION

Periodic cycles have been observed and studied for some time in fungi. The word "circadian" was coined by Halberg (1959) as a term applying to biological wave phenomena or specific phase relationships observed approximately every 24 hours in some organisms. Recent studies have tried to determine if these observed rhythms are exogenous or endogenous in origin.

In the fungus Endothia parasitica, the causative agent of chestnut tree blight, an apparent circadian rhythm is manifested by zonation or growth bands formed by a growing colony. Depending on the type of agar used, these bands are formed by pigmentation of the matrix or else by alternating zones of matrix and aerial hyphae.

Basic studies concerning periodic growth of fungi were started just after the turn of the century. Bisby (1925), working with Fusarium discolor sulphureum, stated that light localized conidia production, therefore causing zonation. Early researchers cited by Bisby, such as Steven and Hall recorded light as the stimulus for zonation patterns; whereas Milburn related the cause to the type of medium used. Gallemaerts was cited as finding that the alternation of light and dark periods was responsible. Brown (1925) and Snyder and Hansen (1941) found light alternation the initial factor in their test organism Fusarium. Dickson (1939) used alternating light and dark periods of equal length in his growth studies on Sclerotinia fructigena and found slight zonation.

The majority of workers interested in the effects of light upon growth responses in fungi have employed visible light only. Experimentation with ultraviolet light started with investigators such as Weston and Halman (1930) who suggested that ultraviolet light acted as a growth stimulus. In 1945 Hollaender and Emmons used ultraviolet irradiation on Neurospora crassa to study mutation effects. Seven wavelengths were utilized, ranging from 228 to 296 nm. Much of the work in the later 1940's was concerned primarily with fungal mutation by ultraviolet light.

The development of the race tube technique by Ryan, Beadle, and Tatum (1943) has greatly facilitated zonation studies. For example, Brandt (1953) used a single 60-watt blue bulb as the only light source while working with N. crassa. He found that high light intensity inhibits zone formation. Zonation was observed in one strain of N. crassa but not in other strains of the same species. In 1959 Pittendrigh et al., elaborated on Brandt's research using the same organism, agar, and the race tube technique. He stimulated growth by using a 14-watt cool white fluorescent lamp for the first 40 hours after inoculation. Following exposure to the fluorescent lamp a red lamp was employed for illumination for the remainder of the experimental period. Pittendrigh observed regular zonation approximately every 22 hours at two different temperatures, 24°C and 31°C. Bianchi (1963) continuing the zonation study of N. crassa, used a cool white fluorescent lamp with gelatin filters (yellow, red, and blue) over the race tubes in experimentation with wavelength response. Cultures grown under the three different filters showed the same pattern of growth and zonation for each.

A literature review showed a lack of work in the near ultraviolet

range as well as limited investigation with blue light.

There is a zonation response in growths of E. parasitica on agar similar to that observed by Bianchi with N. crassa and conjectured by him to be endogenous. I designed, then, a series of experiments with E. parasitica to test the validity of this hypothesis. The objective was to show a relationship between growth zonation in E. parasitica and light, either in continuous or intermittent doses, at various wavelengths.

Following Pittendrigh's work, my study attempted to determine if a single spectral band is the stimulus for the observed circadian rhythm in E. parasitica. Three wavelength regions of the spectra were used with maxima at 365 nm, 375 nm and 450 nm. Far ultraviolet light was not considered because of its mutation effects.

CHAPTER II

MATERIALS AND METHODS

Source of Culture and Inoculation

A stock culture of Endothia parasitica was obtained from the American Type Culture Collection where it is listed as ATCC No. 9414. Endothia parasitica is a recognized plant pathogen which must be disposed of by standard autoclaving techniques.

Inoculation was accomplished by a punching technique. A sterile number two cork borer (0.5 cm in diameter) was used to cut uniform size plugs from the matrix of a standard stock culture, which was at least 2 weeks old. After excess agar had been removed from the plug with a sterile scalpel, it was then placed in the center of the sterile agar in a petri dish.

Two standard mycological agars were used; Sabouraud dextrose and Potato dextrose, both obtained in the dehydrated form from Difco Laboratories. All agar was prepared according to the manufacturer's instructions, employing standard microbiological techniques. Sterile agar was poured into sterile disposable plastic petri dishes (100 x 15 mm), using a quantity of agar sufficient to half-fill each dish. The agar was allowed to solidify in these dishes before use.

After inoculation each petri dish was marked indicating the type of agar and conditions of light exposure. To prevent dehydration all petri dishes were placed in Mylar bags, (five mil thick) usually two plates to a bag, side by side. The ends of the bags were closed with a rubber

band. The inoculated surface always faced upward under all environmental conditions.

Light Rack and Lights

A light rack was built to provide a simple frame support for the lights and to hold them at a fixed distance from the inoculated petri dishes. This arrangement also minimized light escape into, and from the laboratory room. Partitions between the areas illuminated by the different lamps assured no mixture of the different lights.

The light rack, 21" x 49½" x 52½", was constructed with 2" x 4" lumber to support three identical fluorescent, hooded light fixtures. Each light fixture accomodated three 48 inch, 40 watt, T-12 rapid-start bulbs. After the bulbs were installed, the light fixtures were placed on top of the light rack parallel to the rack's width. The bottom of the lamps were 22 inches from the floor of the light rack where the petri dishes were placed.

Black polyethylene plastic, four mil thick, was stapled around all four sides of the light rack to minimize light escape and entrance. A three and one half inch margin was provided from the bottom of the plastic drape to the floor of the light rack; this aided in air circulation. A final outer perimeter wall of heavy cardboard, ten inches high, was stapled to a two inch lateral extension of the light rack floor. This eliminated any light leaks, but did not inhibit air movement. A curtain of black polyethylene plastic was used as a separator between each light fixture. The curtain ran the full length of the light rack, but came to within only two inches of the rack floor. The purpose again was to decrease heat buildup by allowing as much air circulation as possible.

Three types of fluorescent lamps were utilized. The blue fluorescent lamp (B 40-W T-12) had the widest wavelength range from 340 to 680 nm with its maximum output at about 450 nm. The black light (Bl 40-W T-12) covered from approximately 280 to 470 nm with its maximum output at about 375 nm. The narrowest wavelength was developed by the black light with an integral filter (BlB 40-W T-12) ranging from 310 to 420 nm, and had a maximum output at 365 nm. All three lamps are manufactured by General Electric, Sylvania, and Ken-Rad.

Environmental Conditions: Sunlight and Darkness

In addition to the employment of the three light sources previously described, it was necessary to establish a control group that excluded any influence of light. It was also advantageous to create a separate group that would be stimulated by natural sunlight responses.

The group exposed to sunlight was designated as "natural response group" because the rhythmic pattern of light alternation caused by the earth's rotation is more natural than one that could be designed in the laboratory. In the sunlight group inoculated petri dishes in their Mylar bags were placed next to a window having a southern exposure.

The dark or control environment was obtained by the use of a 32 cubic foot incubator made by National Appliance. A single experimental run consisted of leaving inoculated petri dishes undisturbed in their Mylar bags for eight days.

Percent Transmittance, Temperature, And Light Intensity

In order to determine the actual effect of light on E. parasitica, the percent transmittance of the different materials used was measured with a Model 124 Coleman double beam spectrophotometer.

The Mylar bag proved highly transparent to the wavelengths of light employed in this study. It transmitted 100 percent from 750 nm to 310 nm where it then immediately dropped to 9 percent transmittance at 300 nm.

The lid of a previously unexposed clear plastic petri dish transmitted 80-85 percent from 750 nm to 370 nm. A gradual decline followed: 70 percent transmittance at 320 nm, 50 percent transmittance at 308 nm, and essentially zero transmittance at 294 nm. An identical lid which had been exposed to the black light with the integral filter for eight days showed no more than a 5 percent difference compared to the unexposed lid. The results proved that long exposure to ultraviolet light failed to produce a significant difference in the percent transmittance of a plastic petri dish.

Both Sabouraud dextrose and potato dextrose agars were measured for their light absorbance. Because of their gradual increase in absordtion to almost 100 percent in the near ultraviolet compared to air, the results are given graphically (Figure 1). Air was used as the calibrating blank in all spectrophotometer measurements. These spectra indicated considerable adsorption by the media in the blue range as well as the near ultraviolet. The absorption is somewhat greater for the Sabouraud dextrose agar in the visible range. Any absorption for either agar in the near infrared is insignificant.

Temperature recordings were taken during each experimental run. Thermocouples recorded temperatures at different sites under each of the

three hooded light fixtures. Once the overall environmental temperature for the lighted area was known the incubator for dark conditions was adjusted accordingly. Since there was no feasible way to regulate temperature for the group under sunlight conditions, a thermometer recorded the daily changes. However, due to continuous air conditioning, the temperature remained somewhat constant. Temperature averages and ranges are presented in Table 1.

Light intensity was measured in foot-candles with a Model 200 "Lightmeter" produced by Basic Science Industries. A series of colored filters as indicated in Table 2 was used to obtain intensity readings in specific wavelength bands. Using the light meter by itself and with each of the three filters, measurements were taken under the light rack at the level at which the petri dishes were exposed. The results justify the specifications issued by the individual light manufacturers. The blue lamp emits most of its light in the visible range with very little, if any, in the infrared. The black lamp emits some light in the blue-green range but little in the near infrared. The black lamp with the integral filter emits little in the blue-green range and none in the higher visible range. According to the manufacturer's specifications both black lamps emit strongly in the near ultraviolet (365 nm). No device was available for measuring the absolute intensity of these lamps. However, an approximate point of reference can be obtained from Ultraviolet Photobiology which states that two 15 watt black lamps (B1B) produce 20 erg mm^{-2} sec^{-1} at a distance of 50 cm (Jagger, 1967).

Analysis of Cultures

Results were obtained by measuring the diameter of the growth

region around the culture plug after eight days under each environmental condition. Actual measurement was accomplished by placing an accurate millimeter scale across the surface of the growth.

In the case of petri dishes containing Sabouraud dextrose agar the number and shape of the concentric aerial hyphae rings were compared against the controls and the natural response group. With the plates containing potato dextrose agar, the degree of pigmentation and the regularity of growth were used as the comparative values.

A more detailed analysis is presented in the following sections.

Table 1. Temperature Recordings of the Experimental Groups.

Light Conditions	Average Temperature	
	Lamps On	Lamps Off
a) Floor of light rack:		
1) Blue light	31°C	25°C
2) Black light	31°C	25°C
3) Black light with integral filter	30°C	25°C
b) Potato dextrose agar in petri dish enclosed in Mylar bags:		
1) Blue light	31°C	25°C
2) Black light	31°C	25°C
3) Black light with integral filter	30°C	25°C
c) Top of hooded light fixture:		
1) Blue light	31°C	25°C
2) Black light	33°C	25°C
3) Black light with integral filter	33°C	25°C
d) Internal environment of dark group (incubator):		30°-32°C
e) Laboratory room containing sunlight group:	22°-25°C	

Table 2. Light Intensity Recordings for the Hooded Fluorescent Lamps.

Intensity Readings	Dominant Wavelength
Filter	
a) Blue*	425 nm
b) Green-wratten 58B	538 nm
c) Red-wratten 25	617 nm

Measurements in foot-candles		Filters		
Lamp**	Meter	Blue	Green	Red
a) Blue, B 40-W T-12	100	77	23	5
b) Black, Bl 40-W T-12	64	53	6	1
c) Black with integral filter, BlB 40-W T-12	26.5	23.5	0	0

* The blue filter was manufactured by Leitz, the others by Eastman Kodak.
**The lamps were manufactured by General Electric, Ken-Rad and Sylvania.

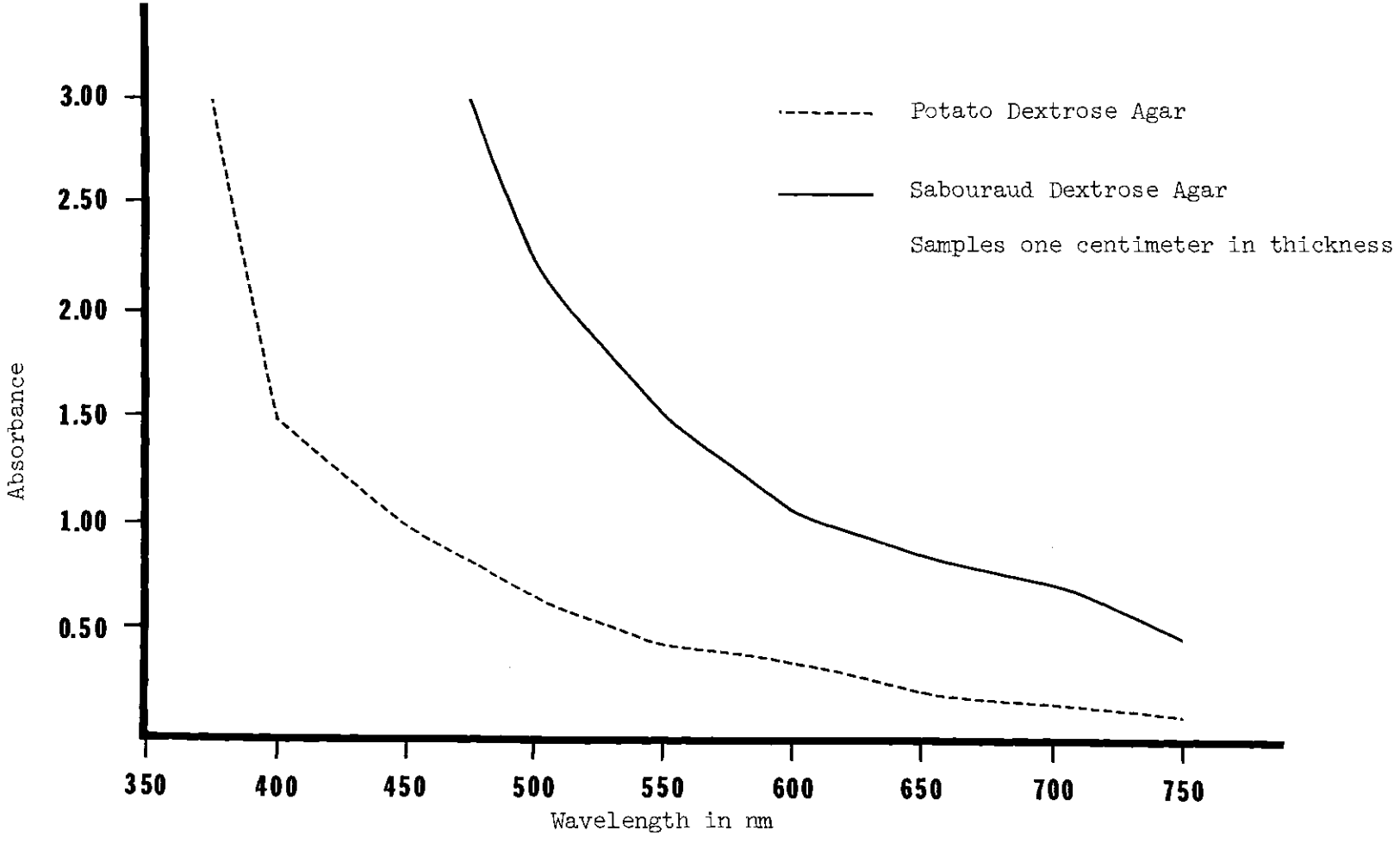

Figure 1. Absorbance of Potato Dextrose Agar and Sabouraud Dextrose Agar at Various Wavelengths of Light.

CHAPTER III

RESULTS

The results are presented in Table 3 through Table 5. Except for a few definitions given here the tables are self-explanatory.

The total number of plates exposed to each condition is indicated by "N" in the tables. These plates were exposed in groups of 6-8 for each run, and a value of a greater than 6-8 indicates duplicate or triplicate runs.

The "total growth diameter" is the total diameter measurement of a culture after eight days under one of the environmental conditions. Diameters of cultures were always measured at their widest points.

The "leading edge" is a growth region around the periphery of a colony and consists entirely of subsurface growth, i.e. it is made up of mycelia growing beneath the agar surface. The width of the leading edge, as described, is a function of environmental conditions.

The "number of aerial hyphae rings" were recorded along with their specific characteristics. The maximum number of rings present under "natural" conditions was six. A ring is formed each day but it takes 48 hours after inoculation before visible growth is apparent.

The term "pigmentation" refers to the coloration of the matrix and aerial hyphae when present.

Table 3. Effect of Light and Darkness on E. parasitica Duration: Eight days

Day-Night (Natural Response Group) Temperature Range: 22-25°C		Continuous Darkness Temperature: 30°-32°C
Potato Dextrose Agar	N = 22	N = 23
Total Growth Diameter (Average):	5.9 cm	5.9 cm
Leading Edge (Average):	0.3 cm	1.3 cm
Pigmentation:	Matrix: Light tan with green. Alternating colors produce zonation. Underside of matrix light and dark tan.	Pigmentation appears sparsely spread over the agar. For the most part the matrix has a white coloration to it.
Growth Patterns:	Growth regular in shape. Typical radiating pattern. Four-six zonation bands. Matrix level.	Regular shape. Radiating line pattern. Two coloration zones or bands present.
Sabouraud Dextrose Agar	N = 20	N = 20
Total Growth Diameter (Average):	5.2 cm	5.1 cm
Leading Edge (Average):	0.1 cm	0.9-1.1 cm
Pigmentation:	Matrix: Tan with white areal hyphae.	Light tan matrix. Aerial hyphae white. Underside of matrix light in color.
Number of Aerial Hyphae Rings:	5-6 complete rings. Regular in shape. Well spaced concentric pattern.	No complete ring or zonation pattern between matrix and aerial hyphae.
Growth Patterns:	Level matrix. Uneven alternating pattern of matrix and aerial hyphae. Aerial hyphae bands wider than matrix bands. Matrix has regular circular shape.	Level matrix. Irregular shape. Greater portion of growth made up of aerial hyphae.

Table 4. Effect of Intermittent Light on E. parasitica Approximately 24 Hour Cycles
Duration: Eight Days

Blue Light (B 40-W T-12)	Black Light (BL 40-W T-12)	Black Light/Filter (BLB 40-W T-12)
Temperature: 25°-31°C	25°-31°C	25°-30°C
Potato Dextrose Agar: N = 24	N = 25	N = 21
Total Growth Diameter (Average): 5.2 cm	4.4 cm	4.8 cm
Leading Edge (Average): 0.9 cm	0.7 cm	0.5 cm
Pigmentation: Matrix: Dark tan with lighter edges. Underside of matrix tan.	Matrix: Dark tan center, lighter on the edges. Underside of matrix tan.	Matrix: Dark tan with lighter on the edges. Underside of matrix tan.
Growth Pattern: Regular growth. Two zonation bands. Matrix level.	Irregular shape. Four complete bands of zonation. Matrix level.	Irregular shape. Three complete bands or zonations.
Sabouraud Dextrose Agar: N = 23	N = 20	N = 21
Total Growth Diameter (Average): 4.4 cm	3.9 cm	4.2 cm
Leading Edge (Average): none	none	none
Number of Aerial Hyphae Rings: 4 complete rings.	4-5 complete rings.	3-4 complete rings.
Pigmentation: Matrix tan. Aerial hyphae white outer rings, yellow inner rings.	Matrix dark tan. Aerial hyphae white outer rings, yellow inner rings.	Matrix dark tan. Aerial hyphae white outer rings, yellow rings.
Growth Pattern: Regular growth. Well shaped concentric circles. Alternation of aerial hyphae and matrix in equal bands. Level matrix.	Irregular in shape. Dried appearance. Matrix not level, compact	Regular in shape. Circular concentric rings. Dried appearance. Level matrix. Alternation of matrix and aerial hyphae. Not in even bands or zones.

Table 5. Effect of Continuous Light on E. parasitica Duration: Eight Days

Blue Light (B 40-W T-12)	Black Light (BL 40-W T-12)	Black Light/Filter (BLB 40-W T-12)
Temperature: 30°-32°C	30°-32°C	30°-32°C
Potato Dextrose Agar: N = 24	N = 25	N = 24
Total Growth Diameter (Average): 5.3 cm	3.1 cm	4.2 cm
Leading Edge (Average): 1.1 cm	0.7 cm	0.4 cm
Pigmentation: Matrix: tan at center getting lighter at the edges.	Matrix: Darker tan than under blue light conditions, but still lighter at the edges.	Matrix: Dark tan at center, lighter at the edges.
Growth Patterns: Radiating growth. Level matrix, brassy appearance of underside of matric. Concentric overlap of matrix could be considered as one growth zonation.	No radiating pattern. Matrix not level, dried appearance, two zonation rings.	Radiating pattern, concentric overlap shows 3 rings. Level appearance and dried.
Sabouraud Dextrose Agar: N = 23	N = 24	N = 21
Total Growth Diameter (Average): 4.1 cm	3.1 cm	3.3 cm
Leading Edge (Average): 0.2 cm	0.7 cm	0.2 cm
Number of Aerial Hyphae Rings: A total of 5-6 rings only the first one complete.	Only 1 complete ring if at all. Most rings not complete, usually only 1 present.	At least 1 completed ring usually 2 or more present.
Pigmentation: Matrix: Tan, outer rings of aerial hyphae white, inner ones yellow.	Matrix: Dark tan with lighter edges. All aerial hyphae yellow in coloration.	Matrix: Dark tan with lighter edges. All aerial hyphae yellow.
Growth Patterns: Ring regularly shaped, level matrix, even zonation between concentric rings. Brassy coloration only under punching.	Rings irregular in shape. No even zonation between aerial hyphae and matrix. Dry appearance, brassy coloration on underside.	Rings regularly shaped, even zonation between aerial hyphae and matrix somewhat dried appearance.

CHAPTER IV

DISCUSSION

Data collected under the topics of "total growth diameter," "leading edge", and "pigmentation" did not furnish information significant enough for analysis. However, they are presented to provide additional information if this line of experimentation is ever elaborated.

The area of paramount interest was that of zone formation. The main purpose of this research was to demonstrate, if possible, a circadian rhythm in E. parasitica through its ability to produce zones or bands in its growth pattern under the influence of periodic changes in incident light intensity.

The results of this zonation study are summarized in Table 6. Only cultures grown on Sabouraud dextrose agar are examined in detail because zonation of cultures grown on potato dextrose agar is difficult to determine accurately. The characteristic appearance of aerial hyphae made zonation easily distinguishable on Sabouraud dextrose agar. Although zonation was exhibited on potato dextrose agar as areas of varying density of matrix, quantitative determination of the exact extent of zonation was considered impossible.

Zonation as it appears on Sabouraud dextrose agar seems to be a two-step process. The first step is an outward growth of subsurface hyphae which is then covered by a surface matrix. Once matrix development has progressed to some set limit (determined by unknown factors but often associated with a dark period) aerial hyphae begin to grow concurrently

with the matrix. This appearance of aerial hyphae constitutes the second step. The matrix continues to spread across the surface of the agar while the aerial hyphae grow above it. After a period of time the matrix will then continue to grow without the formation of aerial hyphae. The growth cycle is then repeated.

For the purpose of this discussion it is assumed that the only variable among the results obtained employing the three fluorescent lamps was the wavelength of the emitted light. Relative intensities were disregarded due to the extreme difficulty of their measurement. Results show that the blue lamp and the black lamp stimulated the largest number of zones formed for continuous and intermittent illuminated groups, respectively. (Table 6). It should also be noted that the black lamp had the smallest number of zonations under continuous conditions whereas the blue lamp was second in the intermittent group. One could conjecture that the black lamp emits light whose wavelength is a stimulator for matrix formation but an inhibitor for aerial hyphae development, based on the theory that zonation is a two-step process (Brandt, 1953). It is possible, but not proven, that the peak wavelength of 375 nm performs this function since the relative maximum output of the lamp appears at that wavelength.

Under intermittent illumination there was zonation with complete ring formation. The matrix growth was stimulated by the black lamp during the 24 hour cycle, while aerial hyphae grew during the 12 hour dark period of that cycle.

The situation is completely reversed under continuous illumination. The black lamp which presumably only stimulates matrix production stimulates only a compact matrix. Only a single ring of aerial hyphae developed.

Lamps having relatively less of the 375 nm wavelength in their spectrum showed a larger number of zonations. Although ring formations of aerial hyphae were not always complete they were present.

Cultures grown in complete darkness showed no zonation and little exposed, pronounced matrix with predominantly aerial hyphae formation.

The mechanism involved appear to be that the black lamp stimulates only surface growth but not above-surface growth, such as aerial hyphae.

Another possibility for the stimulation of zone development seems to be alternating light and dark periods. Bisby (1925) found that periods of exposure as short as two minutes with a tungsten-filament lamp was enough to induce zonation. Brandt (1953) sets forth a hypothesis to explain zonation by the alternating light, using light (wavelength not specific) as an inhibitor of a chain of events. In 1965 Jerebzoff stated that zonation was not found in fungi grown under uniform physical conditions (presumably referring to light). However, I must agree with Pittendrigh (1959) who stated that the occurrence or non-occurrence of zonation does not actually prove the existence of a circadian rhythm but only a connecting or nonconnecting of the biological clock to this particular response.

The results reported here strongly indicate the possibility that specific wavelengths of light as well as alternation of light-dark play an important role in the formation of zones by cultures of E. parasitica. If this is indeed the case, it is possible that this is true also for other similar fungi, and warrants a re-examination of the mechanism of zonation in these terms.

Table 6. Summary of Zonation Results Concerning Cultures Grown on Sabouraud Dextrose Agar. Duration: Eight Days.

Lamps are listed under the experimental conditions with the one having the greatest zonation stimulation first and in decreasing order thereafter.

		No. of Zones	Temperature	Dominate Wavelength (nm)
A.	Intermittent Light (Approximately 24 hr. cycles)			
	1) Black Lamp (BL 40-W T-12)	4-5	31°C	375 nm
	2) Blue Lamp (B 40-W T-12)	4	31°C	450 nm
	3) Black Lamp/Filter (BLB 40-W T-12)	3-4	30°C	365 nm
B.	Continuous Light			
	1) Blue Lamp (B 40-W T-12)	5-6	31°C	450 nm
	2) Black Lamp/Filter (BLB 40-W T-12)	2	30°C	365 nm
	3) Black Lamp (BL 40-W T-12)	1	31°C	375 nm
C.	Natural Response Group			
	1) Sun Light	4-6	Range: 22-25°C	
D.	Continuous Darkness (Control Group)			
	1) Darkness	None	31°C	

CHAPTER V

CONCLUSIONS

A study of the zonation patterns of E. parasitica grown on Sabouraud dextrose agar for a duration of eight days led to the following conclusions:

1. Zonation in E. parasitica does not appear to be related to any endogenous circadian rhythm.

2. The phenomenon of formation zone by growth in bands does appear to be light related.

3. A lamp whose output wavelength peaks at 375 nm has a particular stimulating effect on matrix growth but acts as an inhibitor for above-surface growth, such as aerial hyphae. It is not possible, however, to be certain that the 375 nm region is the one responsible because of the uncertainty in absolute spectral intensities of the lamp at various wavelengths.

CHAPTER VI

RECOMMENDATIONS

From the results of this investigation I recommend the following for future research in this field:

1. A detailed study of the alternation of light as a zonation stimulator in E. parasitica.

2. An investigation into the variation of wavelengths as well as relative intensities as a possible zonation stimulator.

3. A study on the effects of narrow wavelength bands as compared to broad spectra as a zone stimulator.

Results of such studies as recommended here, would verify or deny the conclusion that particular wavelengths of light are the primary stimulating factor in zone formation in fungal growth, and would lead to a better understanding of the process of growth and development in the fungal colony.

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